The Impact of Pazopanib on the Cardiovascular System.

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Introduction

Most patients with cancer now die from cardiovascular (CV) disease rather than from cancer itself and nearly half of patients with renal cell carcinoma experience CV side-effects from the gold-standard therapy—pazopanib[1-3]. Pazopanib is a tyrosine kinase inhibitor (TKI) that inhibits vascular endothelial growth factor receptors (VEGFRs). The clinical use of pazopanib and other TKIs is strongly limited by their association with serious CV side effects. Although little is known about its mechanism, pazopanib causes serious CV effects in 50% of treated patients, including hypertension (HTN), heart failure (HF) and myocardial ischemia[1-3]. It is imperative to explore the mechanisms by which this life-saving drug induces HTN and HF in order to develop strategies to mitigate CV insult and sustain cancer treatment for as long as possible.

We have created a structural heart disease model in mice which display early signs of HF similar to humans. These mice (cKO mice) lack cardiac βII-spectrin, a cytoskeletal protein. These mice have arrhythmias, spontaneous Ca^{2+} release and abnormal expression/localization of cardiac membrane proteins[4]. Our previous microarray data shows that fibroblast growth factor-2 transcript levels are reduced by >50% in cKO mice.

Additionally, wild type (WT) mice with induced HF and humans with HF or atrial fibrillation show downregulation of βII-spectrin in the heart.

We hypothesize that the hypertensive effects of pazopanib are due to sustained activation of the renin-angiotensin-aldosterone system (RAAS), which may be mitigated by Lisinopril. Furthermore, we hypothesize that mortality will be increased in cKO mice due to baseline deficiency in angiogenic pathways.

Methods

Mice
8-Week-old black male WT mice were orally dosed with 30 mg/kg of pazopanib twice daily for 42 days. WT mice and cKO mice were orally dosed with 100 mg/kg of pazopanib and/or 20 mg/kg of Lisinopril once daily for 22 days; flox mice were used as controls.

Blood Pressure
The CODA system was used to gather non-invasive blood pressure readings once per week.

Electrophysiology
Ventricular cardiomyocytes were isolated from mice by Langendorff preparation and tested *in vitro*.

Mouse Measurements
Heart weight and tibia length were measured immediately after organ removal at the conclusion of the experiment.

Immunoblots
Mouse whole heart lysates were electrophoresed and tested with anti-VEGFR-2 antibody.

Results

Mean arterial blood pressure over 42-day treatment

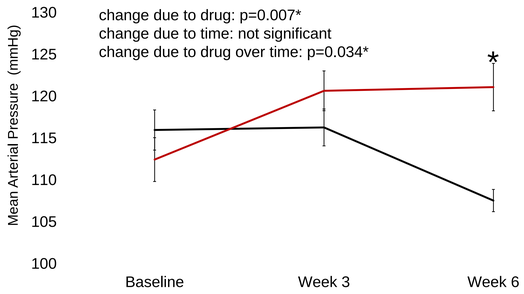
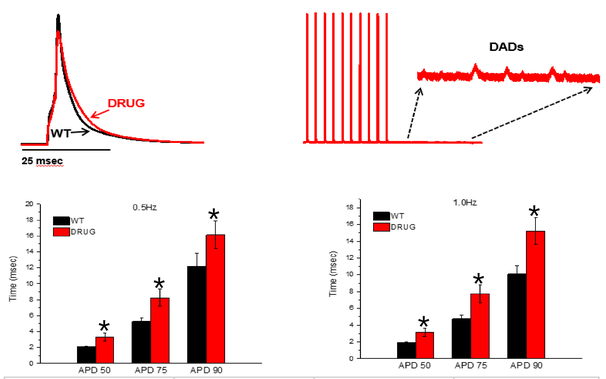


Figure 1. Mean arterial pressure over time of control group (black) compared to mice treated with pazopanib (red). A two-way ANOVA showed a significant increase ($p<0.05$) in mean arterial pressure due to drug treatment but not due to time (n=7). ** indicates statistical significance. Error bars represent ± standard error of mean.*

Electrophysiology results in WT cardiomyocytes after 42-day treatment



Hz/TREATMENT	APD50 +/- SE	APD75 +/- SE	APD90 +/- SE
0.5 WT	2.08 +/- 0.13	5.29 +/- 0.63	12.20 +/- 2.22
0.5 DRUG	3.33 +/- 0.60	8.26 +/- 1.32	16.14 +/- 2.12
1.0 WT	1.92 +/- 0.15	4.77 +/- 0.58	10.11 +/- 1.31
1.0 DRUG	3.13 +/- 0.58	7.74 +/- 1.27	15.20 +/- 1.96

Figure 2. Pazopanib treatment causes precursors to arrhythmias. Average action potential duration (APD) 50, 75 and 90 of myocytes from treated mice (red) was significantly elevated compared to control myocytes (black). 2/6 myocytes from treated mice developed delayed afterdepolarizations (DADs) while these were not observed in 5 control myocytes (n=2).

Mean arterial blood pressure over 22-day treatment

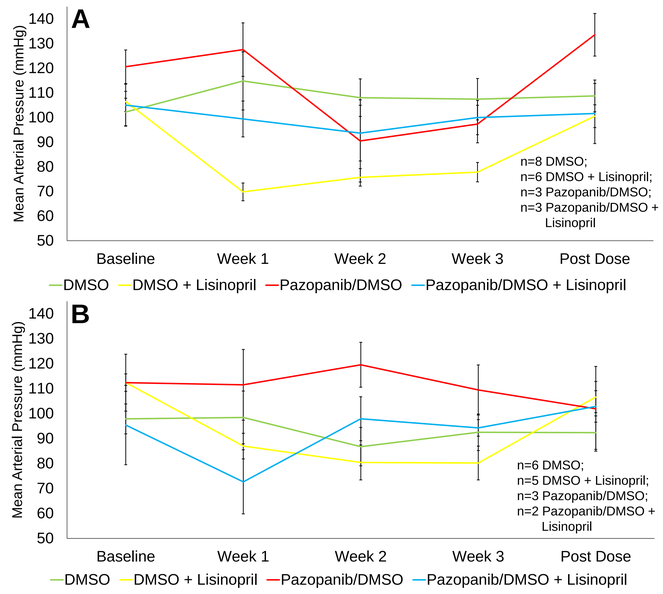


Figure 3. Mean arterial pressure over time of Flox mice (A) and cKO mice (B). The hypertensive effects of pazopanib are more evident in cKO mice (B, red line) at week 2 compared to Flox control mice (A, red line). Lisinopril co-treatment was more effective at attenuating the hypertensive effects of pazopanib in cKO mice (B, blue line) compared to flox control mice (A, blue line).

Survival rate over 22-day treatment

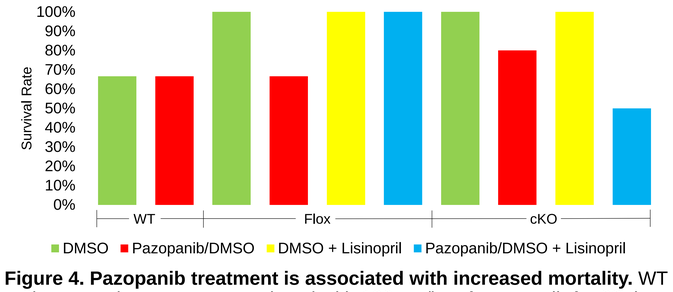


Figure 4. Pazopanib treatment is associated with increased mortality. WT and transgenic mouse groups dosed with 100 mg/kg of pazopanib for 22 days (red) had increased mortality compared to control mice (green). Lisinopril cotreatment decreased mortality in Flox mice only (blue). *n=3 WT DMSO; n=3 WT Pazopanib/DMSO; n=8 Flox DMSO; n=3 Flox Pazopanib/DMSO; n=7 Flox DMSO + Lisinopril; n=3 Flox Pazopanib/DMSO + Lisinopril; n=6 KO DMSO; n=5 KO Pazopanib/DMSO; n=5 KO DMSO + Lisinopril; n=4 Pazopanib/DMSO + Lisinopril.*

Heart weight measurements after 22-day treatment

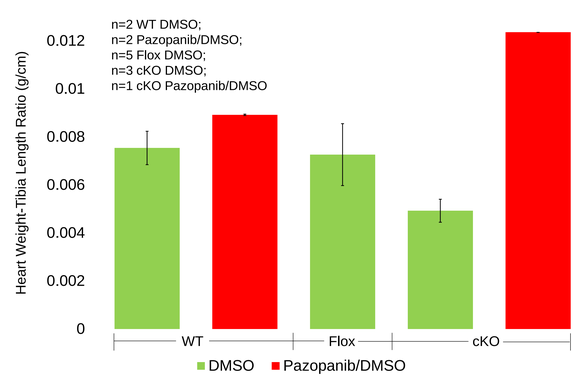


Figure 5. Heart weight-tibia length ratio at conclusion of 22-day treatment. Pazopanib (red) was associated with increased heart weight at the conclusion of treatment compared to control mice (green). Heart weight measurements have yet to be obtained from Flox mice treated with pazopanib.

Discussion

These results support the involvement of the RAAS pathway in the hypertensive effects of pazopanib. We plan to repeat this experiment in order to obtain sufficient *n* values for power. Echocardiography and electrocardiography will be used to asses structural changes in the myocardium as a result of pazopanib treatment. Electrophysiology studies will be performed on cardiomyocytes from mice after 22-days of treatment. Western blotting and microarray analysis will be used to determine which genes are affected by pazopanib treatment.

Our overall goal is to confirm the involvement of the RAAS pathway in the hypertensive effects of pazopanib and the efficacy of Lisinopril co-treatment. Furthermore, we hope to determine whether pazopanib is directly toxic to cardiomyocytes or the vascular endothelium, and whether the cardioprotective properties of Lisinopril are dependent upon its hypotensive effect.

With regards to βII-spectrin, we hope to demonstrate the role of this protein in angiogenic and growth factor pathways, and whether this protein has efficacy as a HF biomarker. Urine and blood samples from mice treated with pazopanib will be analyzed for βII-spectrin breakdown products by western blotting and mass spectrometry.

References

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2. Sternberg CN et al. A randomised, double-blind phase III study of pazopanib in patients with advanced and/or metastatic renal cell carcinoma: Final overall survival results and safety update. *Eur J Cancer*. 2013;49(6):1287-1296.
3. Duffaud F et al. Hypertension (HTN) as a potential biomarker of efficacy in pazopanib-treated patients with advanced non-adipocytic soft tissue sarcoma. A retrospective study based on european organisation for research and treatment of cancer (EORTC). *Eur J Cancer*. 2015;51(17):2615-2623.
4. Smith SA et al. Dysfunction in the βII Spectrin-Dependent Cytoskeleton Underlies Human Arrhythmia. *Circulation*. 131(8):2015;695-708.